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Hypoxanthine levels in amniotic fluid: An indicator of fetal hypoxia?

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During hypoxic conditions intracellular ATP consumption is larger than ATP formation because oxidative phosphorylation is inhibited by the lack of oxygen. The resulting AMP is degraded intracellularly via adenosin and inosin to hypoxanthine. Hypoxanthine passes through the cell membrane into the blood stream. It may be removed in three ways: Further oxidation to xanthine and uric acid in the liver, resynthesis to AMP in the erythrocytes, and direct renal excretion [7]. Since the oxidative degradation of hypoxanthine under hypoxic conditions is decreased, one might expect an accumulation of hypoxanthine in the cells and plasma as well as increased fetal renal excretion into the amniotic fluid.

The determination of plasma hypoxanthine concentrations has been introduced in 1975 by SAUGSTAD [9, 10] into the diagnosis of perinatal hypoxia. Human and animal experiments confirmed an increase of hypoxanthine levels during hypoxia in plasma, CSF, urine and tissue [2, 6, 7, 8, 9, 12, 13]. The goal of this study was to further clarify relations between hypoxanthine levels in the amniotic fluid and fetal hypoxia.

1 Material and methods

Amniotic fluid samples were obtained from 68 pregnant women once (57 cases), twice (7 cases), or three times (4 cases).

Samples obtained during the 16th and 19th weeks of pregnancy were obtained from genetic amniocenteses. All had normal results and a normal subsequent course of pregnancy.

Samples during birth were obtained when intrauterine catheters were introduced to obtain pressure tracings during the first or second phase of labor. In the latter cases the amniotic fluid always had admixtures of blood and mucus.

We considered as normal those deliveries who occurred spontaneously after a normal pregnancy with a duration of labor between four and ten hours, and a one minute APGAR score of the infant of 9 or 10. Gestational duration was determined with ultrasound B-scans [5].

The amniotic fluid samples were denatured within five minutes with a solution of uranylacetate (160 mg uranylacetate in 100 ml normal saline; 1 part amniotic fluid in 10 parts uranylacetate solution). The samples were centrifuged within 20 minutes and the supernatant frozen at - 20 °C for a maximum of six weeks.

Hypoxanthine was assayed according to GARDINER [4]. This method determines hypoxanthine as well as xanthine because it utilizes the release of H_2O_2 following enzymatic conversion of hypoxanthine to uric acid. Chromatographic studies have shown that the proportion of xanthine is low [7]. The accuracy of 20 dual determinations was $s = 2.0$ µmol/l ($\bar{x} = 18$ µmol/l). A tenfold deter-

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mination from a denatured sample was accurate within 1 μmol/l ($\bar{x}$ = 10 μmol/l). Samples from four cases with a total of 14 samples some of which contained small admixtures of blood served to determine that traces of blood do not influence the result ($\bar{x}$ = 7.0 ± 2.3 μmol/l without blood; $\bar{x}$ = 6.3 ± 2.3 μmol/l with trace of blood).

Statistical methods included t-test, WILCOXON test, Chi square test and analysis of correlation [3, 15].

2 Results

Levels of hypoxanthine in the amniotic fluid in relation to gestational age is demonstrated in Fig. 1. There is a slight increase of hypoxanthine levels in normal pregnancies as gestation advances (n = 15).

Hypoxanthine levels increase during birth in amniotic fluid (normal pregnancies at 37–40 weeks of gestation n = 10, $\bar{x}$ = 3.4 ± 0.9 μmol/l; normal delivery at 37–40 weeks gestation n = 19, $\bar{x}$ = 7.0 ± 5.4 μmol/l, $p < 0.02$). Between first ($\bar{x}$ = 6.2 ± 3.4 μmol/l, n = 10) and second stage of labor ($\bar{x}$ = 7.2 ± 6.8 μmol/l, n = 9) there were no significant difference in the levels.

Cases of Rh immunization (n = 5), diabetes mellitus (n = 8), amniotic fluid containing meconium (n = 7), mild hypertension (n = 11), other maternal complications during pregnancy (n = 4), and fetal malformations (n = 2) there were no differ-

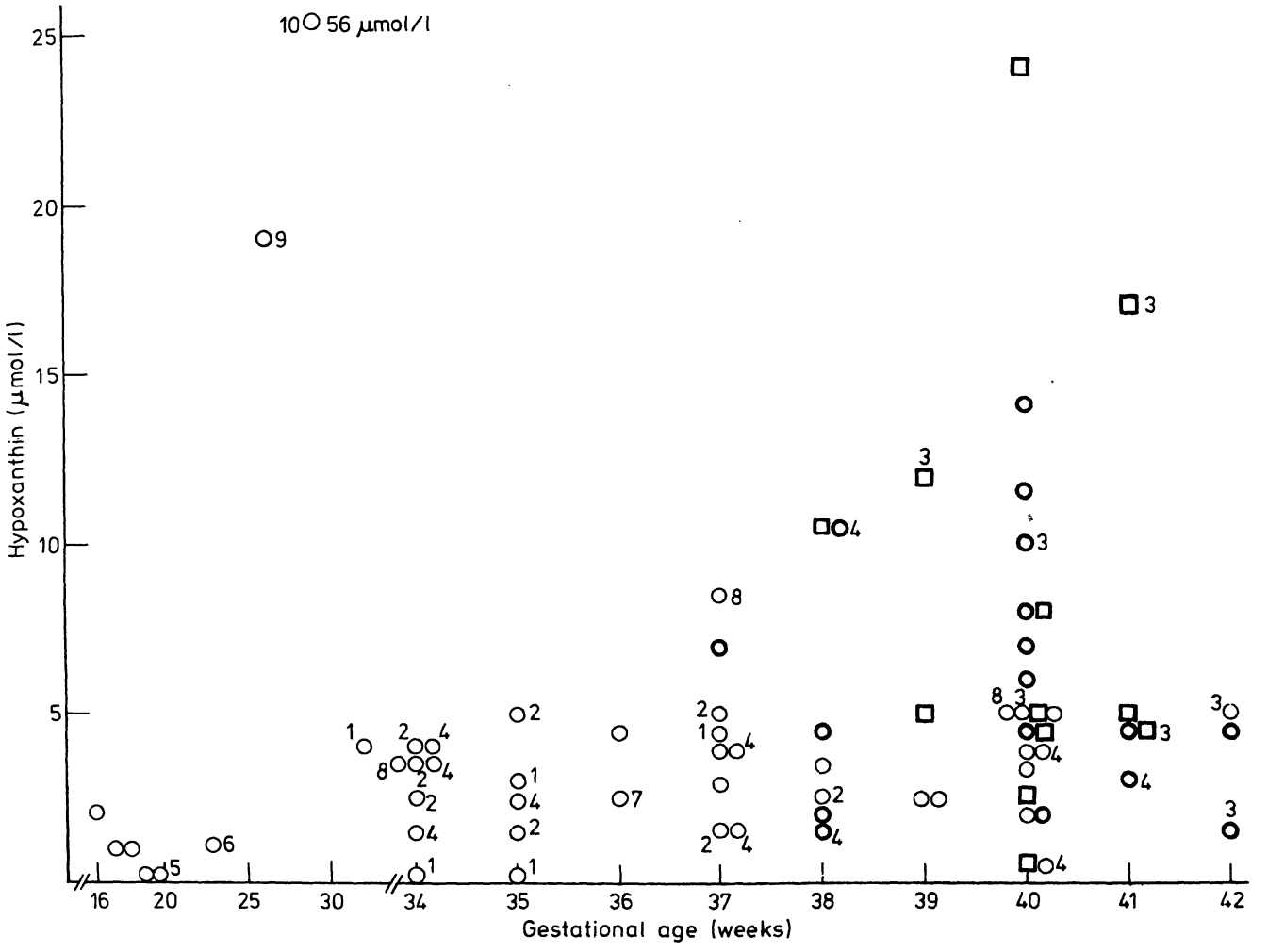

Fig. 1. Hypoxanthine concentrations (μmol/l) in the amniotic fluid in correlation to gestational age and presence of labor in normal and complicated pregnancies.
o (thin circle) = patients not in labor · o (bold circle) = first stage of labor □ (bold square) = second stage of labor
Signs without number: normal pregnancy or delivery
1 = Rh immunization 2 = Diabetes mellitus 3 = meconium stained amniotic fluid 4 = mild hypertension
5 = mitral valve stenosis 6 = anencephaly 7 = anterior duplication anomaly of fetus 8 = maternal hepatopathy
9 = intrapartum fetal death 10 = antepartum fetal death.

ences in the hypoxanthine levels to those found in normal pregnancies and deliveries. Intrauterine fetal death resulted in markedly increased hypoxanthine levels in the amniotic fluid (antepartum fetal death at 29 weeks, 56 μmol/l; intrapartum fetal death at 26 weeks, 19 μmol/l of hypoxanthine). There was a significant correlation between the one minute APGAR scores and the hypoxanthine levels in both the amniotic fluid and the umbilical vein blood in that the hypoxanthine levels were significantly lower with APGAR scores above six than with APGAR scores of six or less (Tab. I). Amniotic fluid values were higher than those in the umbilical venous blood. There was a positive correlation between hypoxanthine levels in the amniotic fluid and in the umbilical venous blood ($r = 0.52$, $n = 25$, $p < 0.05$).

Four newborns with other signs of possible hypoxic events (bradycardia, severe variable deceleration, tight umbilical cord but with normal APGAR scores had hypoxanthine levels in the amniotic fluid of 5, 8, 15, and 16 μmol/l respectively. In one case of severe diabetic fetopathy with acidosis (pH 7.02) the amniotic fluid hypoxanthine levels toward the end of the first phase of labor was 1.0 μmol/l.

There was some correlation between hypoxanthine levels in the amniotic fluid and maternal age ($r = -0.32$, $n = 40$, $p < 0.05$).

No significant correlation was found between hypoxanthine levels in the amniotic fluid and the following variables: maternal hemoglobin level, maternal weight, parity, birth weight, and percentile value of the birth weight.

Tab. I. Hypoxanthine concentration (μmol/l) in amniotic fluid and umbilical vein plasma in correlation to one minute APGAR scores.

APGAR score	Amniotic fluid				Umbilical vein			
	n	$\bar{x}$	S.D.	Range	n	$\bar{x}$	S.D.	Range
1– 2	1	13	–	–	1	18	–	–
3– 6	3	13.1	5.0	7–17	7	4.3	1.8	1–7
7– 8	6	5.5	1.5	3– 9	11	2.3	1.7	0–6
9–10	25	5.8	5.1	0–24	26	2.1	2.0	0–6

Differences for values with APGAR scores 1–6 and 7–10 respectively: Amniotic fluid $p < 0.05$; Umbilical venous blood $p < 0.02$

3 Discussion

Increased hypoxanthine levels in amniotic fluid and umbilical venous blood as a marker of previous hypoxia were found with intrauterine fetal death or depressed newborns with APGAR scores of 1 through 6. However, we could not demonstrate the close correlation between APGAR score, clinical signs of hypoxia, and hypoxanthine levels in either amniotic fluid or umbilical venous blood as had been reported by SAUGSTAD [9].

High concentrations of hypoxanthine in the amniotic fluid apparently may occur during birth as a consequence of labor activity independently from the fetal status. This elevation of hypoxanthine levels is probably caused by the increased release of hypoxanthine associated with uterine contractions [1].

Thus, increased hypoxanthine concentrations in the amniotic fluid may be caused either by fetal hypoxia or by labor activity. Consequently, the determination of hypoxanthine levels in the amniotic fluid cannot be used for the diagnosis of fetal hypoxia.

Summary

During hypoxia there is an increased formation of hypoxanthine from the consumption of ATP; simultaneously the oxidation into uric acid is decreased. The purpose of this study was to determine possible correlations between hypoxanthine concentrations in the amniotic fluid and states of fetal hypoxia.

We obtained 83 amniotic fluid samples from 68 patients during pregnancy or delivery. Hypoxanthine was assayed fluorimetrically according to GARDINER [4].

In the course of pregnancy, hypoxanthine levels in the amniotic fluid rise slightly. A marked increase occurs during delivery (without labor $\bar{x} = 3.4 \pm 0.9$ μmol/l, with

labor $\bar{x}$ = 7.0 ± 5.4 µmol/l, $p < 0.02$). There is no significant difference in the levels obtained from the first and second stages of labor. In intrauterine fetal death (Fig. 1) and in depressed newborns (Tab. I) there were increased hypoxanthine levels in the amniotic fluid, this was not seen in other complications of pregnancy. Independently from the fetal state increased hypoxanthine concentrations may occur in the amniotic fluid evidently as a consequence of labor activity. **Thus the determination of hypoxanthine levels in the amniotic fluid cannot be used for the diagnosis of fetal hypoxia.**

Keywords: Amniotic fluid, fetal hypoxia, hypoxanthine.

Zusammenfassung

Ist die Hypoxanthin-Konzentration im Fruchtwasser ein Parameter fetaler Hypoxiezustände?
Unter hypoxischen Bedingungen kommt es zu einer erhöhten Bildung von Hypoxanthin in Folge des ATP-Abbau, gleichzeitig ist der weitere oxidative Abbau zu Harnsäure vermindert. Das Ziel der vorliegenden Arbeit war es, mögliche Beziehungen zwischen der Hypoxanthin-Konzentration im Fruchtwasser und fetalen Hypoxiezuständen aufzudecken.
Von 68 Schwangeren wurden insgesamt 83 Fruchtwasserproben während der Schwangerschaft oder unter der Geburt entnommen. Die Bestimmung des Hypoxanthin erfolgte fluorimetrisch nach GARDINER [4].
Im Verlaufe der Schwangerschaft steigt die Hypoxanthin-Konzentration im Fruchtwasser leicht an. Ein deutlicher Anstieg findet sich unter der Geburt (ohne Wehentätigkeit $\bar{x}$ = 3,4 ± 0,9 µmol/l, bei Wehentätigkeit $\bar{x}$ = 7,0 ± 5,4 µmol/l, $p < 0,02$). Zwischen Eröffnungsphase und Austreibungsphase besteht kein signifikanter Konzentrationsunterschied. Bei abgestorbenem Feten (Abb. 1) und deprimiertem Neugeborenen (Tab. I) wurden erhöhte Hypoxanthin-Konzentrationen im Fruchtwasser gefunden, nicht jedoch bei anderen Komplikationen der Schwangerschaft. Auch unabhängig vom fetalen Zustand können erhöhte Hypoxanthin-Konzentrationen im Fruchtwasser, offenbar als Folge der Wehentätigkeit, auftreten. **Damit scheidet die Bestimmung der Hypoxanthin-Konzentration im Fruchtwasser zur Diagnostik fetaler Hypoxiezustände aus.**

Schlüsselwörter: Fetale Hypoxie, Fruchtwasser, Hypoxanthin.

Résumé

Concentration d'hypoxanthine dans le liquide amniotique: S'agit-il d'un indicateur d'hypoxie foetale?
En hypoxie la formation d'hypoxanthine augmente du fait de la dégradation de l'ATP, alors que l'oxydation en acide urique diminue. Le but de ce travail est de déterminer les corrélations éventuelles entre les concentrations d'hypoxanthine dans le liquide amniotique et l'hypoxie foetale.
Les auteurs ont obtenu 83 échantillons de liquide amniotique en provenance de 68 patientes au cours de la grossesse ou de l'accouchement. Les dosages d'hypoxanthine ont été effectués par fluorimétrie selon la technique de GARDINER [4].
Pendant la grossesse, la concentration d'hypoxanthine augmente légèrement dans le liquide amniotique. Une élévation importante se produit en cours de travail (en dehors du travail $\bar{x}$ = 3,4 ± 0,9 µmol/l, en cours de travail $\bar{x}$ = 7,0 ± 5,4 µmol/l, $p < 0,02$). Il n'y a pas de différence significative entre les concentrations obtenues en début et en fin de travail. L'hypoxanthinamnie augmente dans les morts foetales in utero (Fig. 1) et en cas de nouveaux-nés déprimés (Tab. I), ce qui n'est pas retrouvé au cours des autres complications de la grossesse. L'augmentation de l'hypoxanthinamnie peut se produire à l'évidence comme conséquence de l'activité utérine, indépendamment de l'état foetal. **C'est pourquoi la détermination de la concentration d'hypoxanthine dans le liquide amniotique ne peut être utilisée pour diagnostiquer l'hypoxie foetale.**

Mots-clés: Hypoxie foetale, liquide amniotique, hypoxanthine.

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